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# Quantitative proteomic analysis of Ibuprofen-degrading *Patulibacter sp.* strain I11


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## Introduction

The increase in diversity and quantity of Pharmaceutically Active Compounds (PhACs) detected in the effluents of wastewater treatment plants is an issue of great concern due to health and environmental associated risks of the PhACs [1].

Ibuprofen, a non-steroidal anti-inflammatory drug, is considered one of the most frequently occurring PhACs in the influent wastewater, typically being found in the range of 10-400 µg/L. Typical Ibuprofen removal efficiencies range from 80-100%, depending on operational conditions and wastewater treatment plant configuration[2,3]. The elimination of ibuprofen is being ascribed primarily to biodegradation. However, in order to investigate the conditions for better removal of compounds like ibuprofen, we need to know the identity of the organisms involved and how their ibuprofen degradation activity depend on the controlling parameters. For this purpose we wanted to identify the genes involved and develop quantitative molecular tools for determining the activity of these genes.

## Objective

The main objective of this study was to investigate the biochemical pathway of ibuprofen degradation in the ibuprofen degrading strain *Patulibacter sp.* Strain I11 using quantitative tandem mass spectrometry.

## Methods

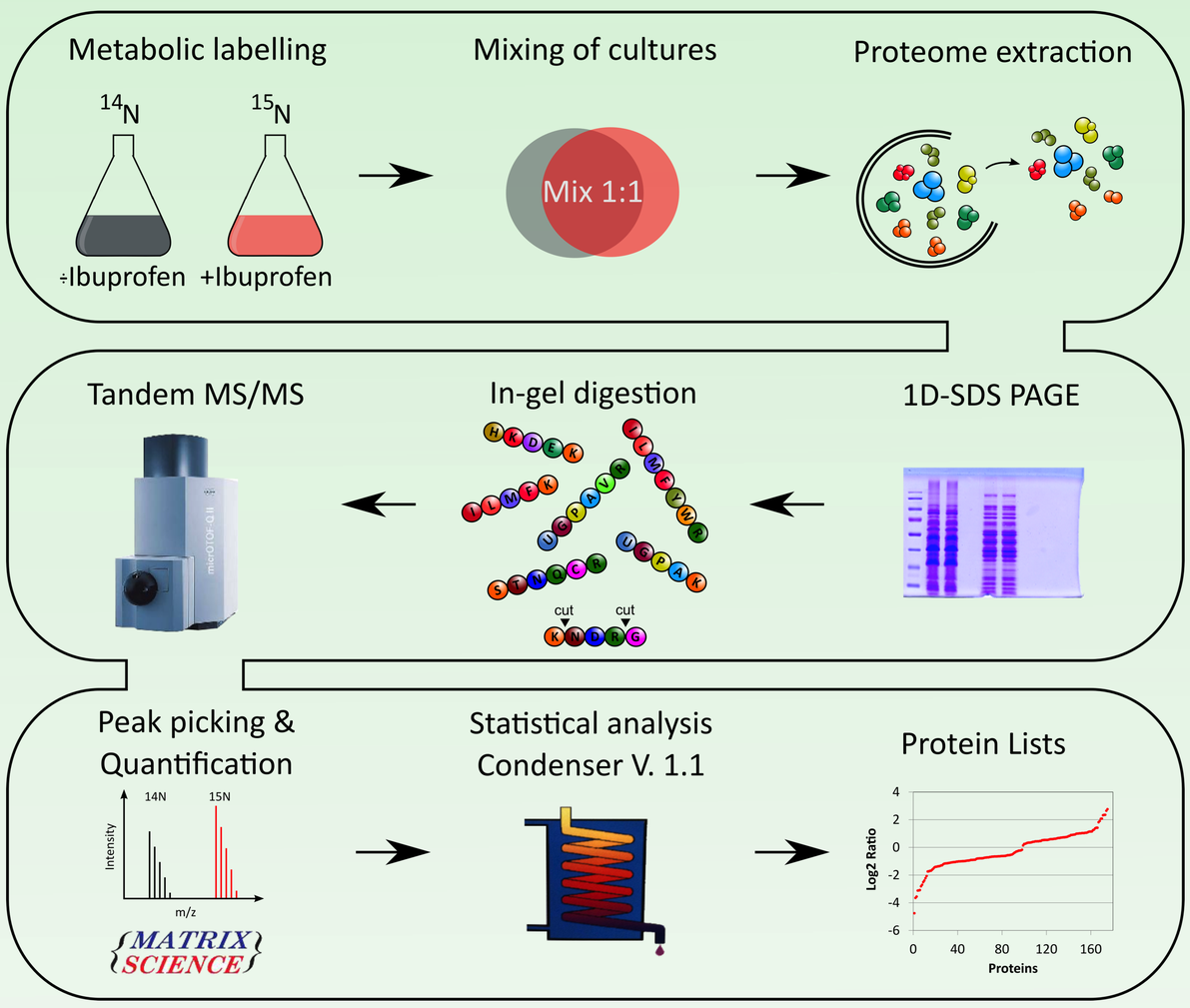



**Fig. 1** The differential changes in the proteome of *Patulibacter sp.* strain I11, grown in the presence and absence of ibuprofen, were characterised by the combination of stable isotope metabolic labelling and 1-D gelbased shotgun Proteomics. The genome of *Patulibacter sp.* Strain I11 was sequenced and annotated and used as the reference database for the subsequent MS-based protein identification (the sequencing and annotation part of the genome of *Patulibacter sp.* strain I11 have been omitted in the above flowchart). The setup was carried out in biological duplicates using a forward and reverse labelling strategy (only the forward labelled duplicate is depicted above). For the reverse labelled duplicate the metabolic labels were reversed, i.e. yielding ($^{14}$N + Ibuprofen) and ($^{15}$N ÷ Ibuprofen). The forward and reverse labelling strategy served the purpose of evaluating potential bias of the $^{14}$N- and $^{15}$N- medium on protein expression levels.

## Results

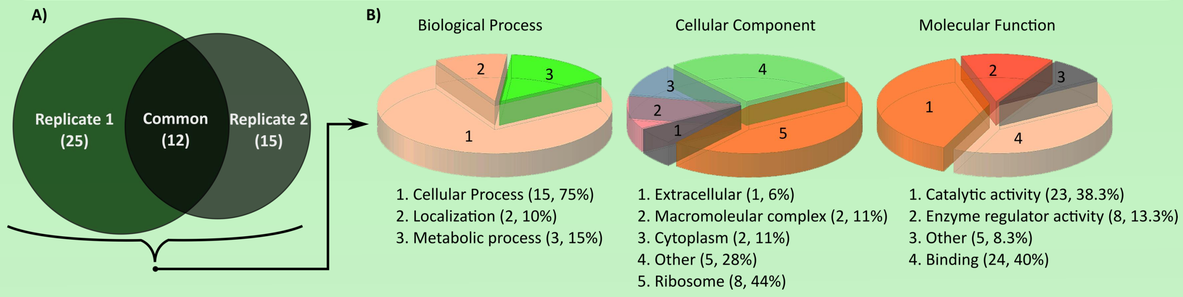
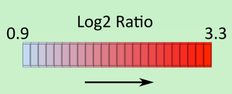



**Fig. 2 A)** Metabolic labelling was carried out in biological duplicate and proteins considered up-regulated (proteins with log2 ratio ≥ 0.9) were **B)** pooled and Gene Ontology-annotated at three different levels: Biological Process, Cellular Component and Molecular Function. Each pie slice is labelled with the GO subcategory name, number of GO annotations within the category as well as the percentage fraction of annotations. Replicate 1 corresponds to the forward labelled replicate (14N ÷ Ibuprofen, 15N + Ibuprofen) whereas Replicate 2 corresponds to the reverse labelled replicate (14N + Ibuprofen, 15N ÷ Ibuprofen).




| | Accession[A] | Description[B] | Log2 Ratio[C] Replicate 1 | Log2 Ratio[C] Replicate 2 | Protein Score[D] Replicate 1 | Protein Score[D] Replicate 2 | #[E] Replicate 1 | #[E] Replicate 2 |
|---|---|---|---|---|---|---|---|---|
| ● | D3F114 | Enoyl-CoA hydratase/isomerase | | - | 145 | | 2 | |
| | D3F2P4 | Nuclear export factor GLE1 | | | 335 | (388) | 17 | (25) |
| ● | D3F178 | Putative uncharacterized protein | | - | 482 | | 24 | |
| | E6SEB9 | Aminotransferase class-III | | - | 96 | | 8 | |
| ● | E1VKI1 | AMP-dependent synthetase and ligase | | - | 314 | | 6 | |
| | D3FA23 | Putative uncharacterized protein | | | 174 | (382) | 6 | (10) |
| | D3F6B1 | Putative uncharacterized protein | | | 311 | (292) | 7 | (14) |
| | Q47N65 | Putative uncharacterized protein | | | 235 | (219) | 4 | (4) |
| | D3F293 | Putative uncharacterized protein | | | 747 | (723) | 40 | (46) |
| ● | D3F3V3 | Rieske (2Fe-2S) iron-sulphur domain protein | | | 313 | (366) | 13 | (13) |
| | D3FFG0 | Putative uncharacterized protein | | | 2195 | (1785) | 45 | (97) |
| | B0CFJ3 | Putative uncharacterized protein | | | 113 | (110) | 4 | (6) |
| | F1YMM4 | Glycosidase | | - | 749 | | 16 | |
| | D3FBK3 | Collagen triple helix repeat protein | | | 412 | (316) | 26 | (21) |
| | D3EZ72 | Putative uncharacterized protein | - | | | (881) | | (46) |
| | D3FEE9 | Putative uncharacterized protein | - | | | (252) | | (8) |
| | D3FEF4 | PpiC-type peptidyl-prolyl cis-trans isomerase | | - | 958 | | 64 | |
| ● | D3FBI3 | Daunorubicin resistance ABC transporter ATPase subunit | | | 509 | (473) | 8 | (10) |
| ● | D3FBH9 | Extracellular solute-binding protein family 1 | | - | 369 | | 8 | |
| ● | D3FBI2 | ABC-2 type transporter | | - | 161 | | 4 | |

**Table 1** Differentially expressed proteins of the biological replicates of *Patulibacter sp.* I11 grown in presence/absence of ibuprofen. Only up-regulated proteins (Log2 ratio ≥ 0.9) are shown in the table. No major influence of the $^{14}$N- and $^{15}$N- medium on the protein expression levels was observed.
[A]UniProt accession number of the closets protein homologue Description of the closets protein homologue, [B]Description of the closest protein homologue, [C]Log2 ratio obtained from the quantitative proteomics analysis, [D]Protein Score obtained from the quantitative proteomics analysis, [E]The number of quantitated peptides upon which the quantitative value (Log2 ratio) was determined.

## Conclusion

- Several proteins related to uptake and degradation of aromatic acids as well as compound transport-related proteins were found among the proteins up-regulated in response to Ibuprofen.
- The high number of up-regulated putative uncharacterised proteins might suggest a novel pathway for the degradation of Ibuprofen in *Patulibacter sp.* Strain I11.

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